AN INVESTIGATION OF CROSS STRIATIONS AND MYOSIN FILAMENTS IN MUSCLE *

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Introduction

Present information on muscle structure is of two rather distinct types, that derived from histological studies through direct observation with the light microscope, and that deduced from indirect methods such as involve polarized light microscopy, streaming double refraction of myosin solutions or x-ray diffraction. Histological studies have always been limited by the inadequate resolving power of light microscopes; the indirect methods have been limited by the fact that they can supply information only in proportion to the correctness of the necessary assumptions. Although valuable knowledge has resulted from both types of approach, there is a lack of definite information on structures below the limit of light microscope resolution. The electron microscope provides the possibility of extending direct observation into a range of dimensions reached hitherto only by indirect methods and conjecture. Since success in biological electron microscopy depends to a large extent on the development of techniques suitable for the material, these initial observations may be expanded as new techniques are developed.

It is impossible in the present space to summarize the previous research on muscle or the ideas put forth concerning its structure; reference to the literature will be limited to those papers which provide a basis for the present study. A general review has been presented by Fenn (1945) while the histological aspects have been reviewed by Jordan (1933). Polarized light investigations have been reviewed by Schmidt (1937) and by Weber (1934). Results of investigations of streaming double refraction in myosin solutions may be found in the papers of Edsall (1930, 1942), von Muralt and Edsall (1930), Mehl (1938) and Dainty, Kleinzeller, Lawrence, Miall, Needham, Needham and Shen (1944). Astbury and Dickinson (1940) and Astbury (1942) described wide-angle x-ray diagrams of muscle and myosin, and Bear (1944, 1945) described small-angle x-ray patterns of various muscles. A discussion of muscle physics has been presented in a review by Ramsey (1944).

In the past, the electron microscope has been used very little in the study of muscle structure. Richards, Anderson, and Hance (1942) have shown electron micrographs of a wedge-cut section of cockroach striated muscle in which each darker band can be seen to consist of three components. Sections of muscle tissue cut by another method were examined by Sjöstrand (1943) but the electron micrographs bear no obvious relation to known structures in muscle. In both these stud-

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ies the object was primarily to investigate sectioning techniques for electron microscopy rather than to study muscle itself.

Ardenne and Weber (1941) published electron micrographs of filamentous myosin particles, which are undoubtedly to be identified with the asymmetrical particles responsible for the streaming birefringence phenomena in myosin sols. Hall, Jakus, and Schmitt (1945) have studied another fibrous protein from molluscan muscles and correlated the structure with the x-ray diffraction results described by Bear (1944).

Electron Microscope Observations of Myofibrils

Preparation of specimens

A primary difficulty in the study of striated muscle with the electron microscope is the preparation of the tissue thin enough to be partially transparent to the electron beam. In view of the difficulties connected with present microtome techniques, a preparative method was sought in another direction. One procedure which has been used previously depends on the tendency of many materials to fragment along natural cleavage boundaries. If such tendency to natural cleavage exists, it may be assisted by chemical or physical means or both. Thus collagenous tissue may be separated into fine fibrils by mechanical "teasing" and the separation is facilitated by weak acid (Schmitt, Hall, and Jakus, 1942). Such methods of fragmentation are applicable to many materials and are readily adapted to striated muscle.

Fresh muscle is fixed in 10 per cent formalin, cut into small pieces and subjected to mechanical agitation in a Waring Blendor. When the resulting suspension is lightly centrifuged to throw down the larger fragments, the supernatant exhibits the characteristic sheen associated with fibrous suspensions. It consists mostly of thin fibrils which may be washed and applied directly to a conventional electron microscope specimen grid with collodion membrane. These fibrils are to be identified with the myofibrils, or sarcostyles, of the muscle fiber.

Most of the observations to be described were made on fibrils from the leg muscles of frog and rabbit. The muscles of lobster and scallop (striated portion) were found to contain relatively large fibrils which were usually quite opaque in the electron microscope. It was necessary to apply fixatives to all these muscles in order to obtain intact fibrils. Wing muscles of the fly, on the other hand, could be teased apart in weak saline solution without previous fixation.

Most myofibrils from frog muscle have widths between 0.5 and 1.0 μ, although a few may be found as wide as 3.0 μ or as narrow as 0.2 μ. They appear to be ribbon-shaped on the electron microscope specimen holder but it is not possible to say whether this shape results from forces produced during drying or whether it represents the form of the myofibril in the intact tissue.

Although most of the structural features of the myofibril can be observed without the use of stain, contrast in the image may be increased by the application of phosphotungstic acid to the specimen (0.1 per cent solution at pH 3–5 for about one minute). The staining procedure is found to be particularly useful in increasing the contrast of the myosin filaments.

An RCA electron microscope Type B with accelerating voltage raised to 65 KV was used throughout the study. A higher voltage would probably be advantageous for this type of material but is not readily obtainable from the standard power supply.

Unstained fibrils

Unstained myofibrils from striated muscle are characterized by a succession of transverse bands of varying density, the main features of which can readily be identified with the bands previously described in histological studies (Jordan, 1933). An electron micrograph of a typical fibril from a slightly stretched frog sartorius is shown in Figure 1, together with the histological designations. The repeating unit, or sarcomere, is bounded terminally by a narrow dense band usually referred to as the *Z* membrane or telophragma. This band is in the center of a light region, the *I* (or *J*) band, so called because it is relatively isotropic. Contiguous with *I* is the sharply-defined *A* (or *Q*) band which is optically anisotropic and has a higher scattering power for the electron beam than does *I*. In some myofibrils the *H* disc (or median disc of Hensen) appears as a lighter region in the middle of the *A* band (Fig. 1). Bisecting the *A*, or the *H* disc if it is present, is the narrow dark *M* band (or mesophragma). There may also appear, in either half of the *I* band, a relatively dark band designated as *N* in Figure 2. These bands are not always present and have been considered by some cytologists to be artifacts resulting from the lining up of granules. In the present study they have been noted in rabbit muscles but are either faint or absent in frog muscles. The clarity of their appearance in rabbit fibrils leaves no doubt as to their existence in this formalin-fixed material.

Myofibrils from rabbit muscle usually show two to four fine cross bands symmetrically disposed with respect to the *M* band and near it. They are beyond light microscope resolution, being about 0.1 μ from center to center, and represent a periodic variation in the dense material of the *A* band.

In favorable cases fibrils can be seen to consist of longitudinal myosin filaments which are difficult to resolve in unstained fibrils except where they fray out at the edges. They are visible in various parts of Figures 1 and 2.

Stained fibrils

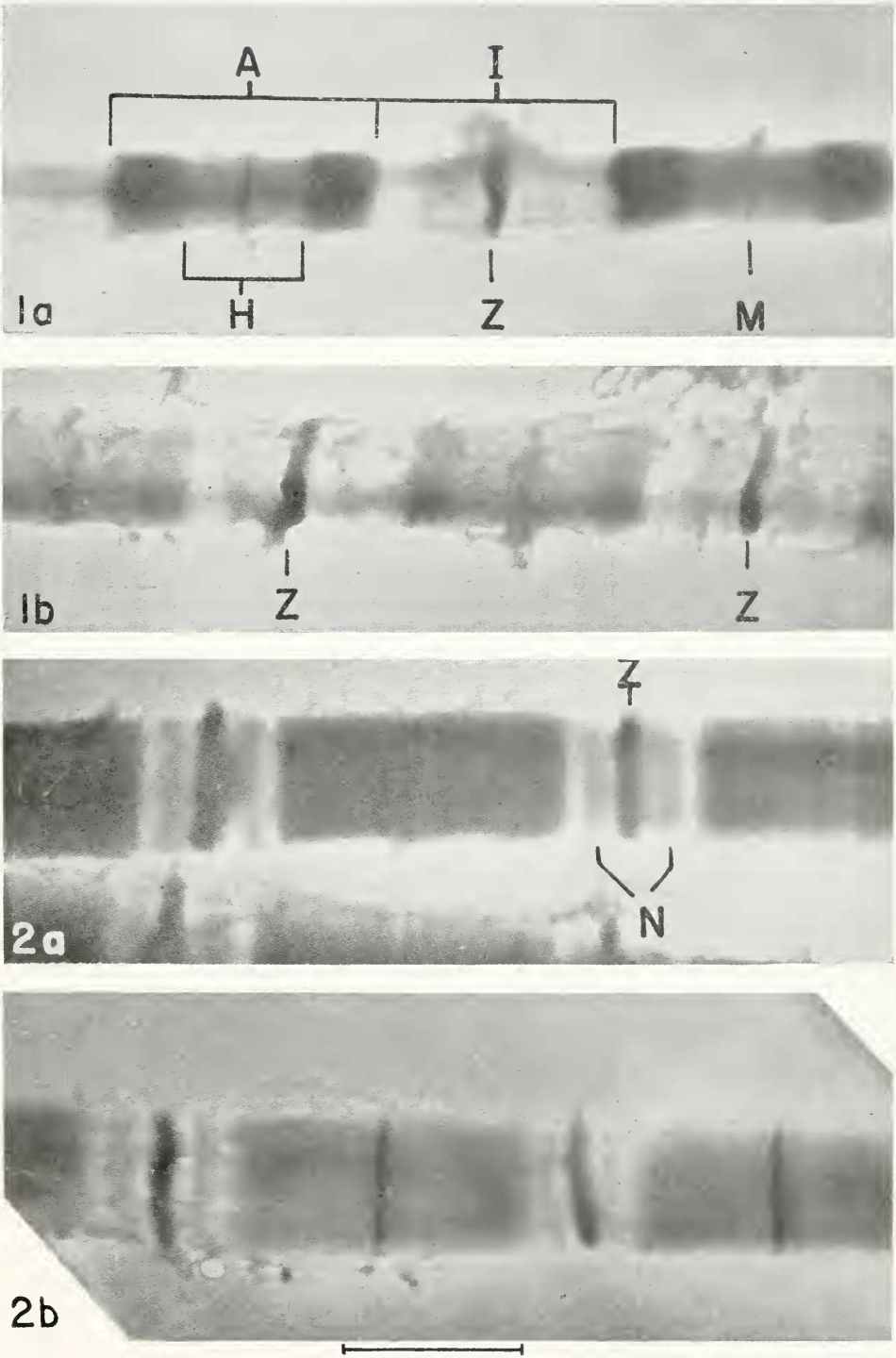
Phosphotungstic acid combines with the cross bands roughly in proportion to their intrinsic density and serves to increase the contrast of the structures described above. Segments of stretched myofibrils stained with phosphotungstic acid are shown in Figure 3. The *A* band is stained more heavily than is the *I* band, and the *M* and *Z* bands appear quite opaque. When the *H* disc is present, it absorbs less stain than does the remainder of *A*.

Besides accentuating the transverse bands, phosphotungstic acid enhances the contrast of the longitudinal myosin filaments. These range in width from 50 to 250 Å and extend continuously, and in relatively straight lines, through both *A* and *I* bands. Although the filaments are usually indistinguishable within the dense *Z* bands, they can be traced through several successive sarcomeres when the *Z* bands are partially disintegrated. In the *A* band the filaments are relatively dense, sharply defined, and almost parallel in orientation. In the *I* band they are less perfectly aligned and of lower density. The higher density of the *A* band appears to result from the higher density of the component filaments.

FIGURE 1. Myofibrils from frog sartorius, stretched about 30 per cent, unstained. × 25,000. All myofibrils shown in this and subsequent figures were fixed in 10 per cent formalin.

FIGURE 2. Myofibrils from rabbit leg muscle, unstained. × 25,000.

PLATE I



In both bands, the myosin filaments present a knotted or beaded appearance with frequent constrictions and variations in density. The nodes are often almost equidistant along the filament and, in some regions of the fibril, they may be aligned laterally to produce a fine cross striation with a period of about 400 Å in the direction of the fibril axis. However, this feature has not been found sufficiently reproducible or regular to be designated as a periodic spacing in the nature of those found in collagen and in clam muscle fibrils.

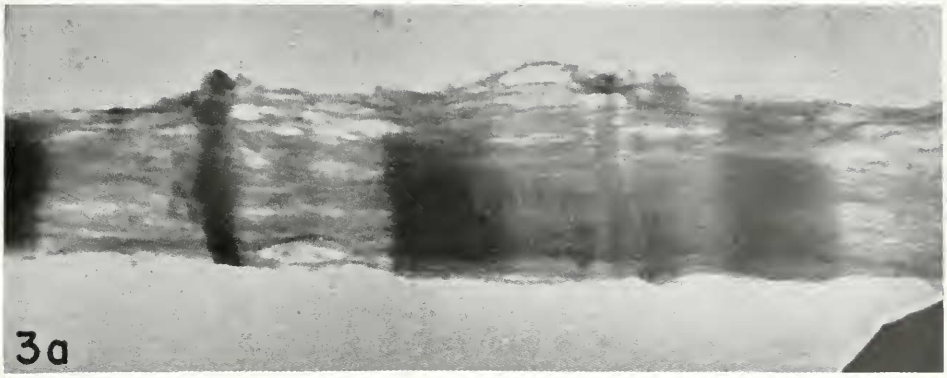
The higher density of the *A* band in electron micrographs of unstained fibrils is in accord with the fact that the refractive index of *A* is higher than that of *I*. Since the myosin filaments pass continuously through both bands, it may be concluded that some other substances occur in much higher concentration in *A* than in *I*. This conclusion is in agreement with the observations of Scott (1932), who found a higher concentration of salts in the *A* band, and of Macallum (1905), who demonstrated a distribution of potassium within the *A* band which is remarkably like the distribution of unstained density as seen in Figure 1. Thus it seems quite evident that the *A* band contains, besides myosin filaments, some substance characterized by a relatively high concentration of salts. This material will be designated as the "*A* substance". Phosphotungstic acid is apparently absorbed in proportion to the density of the *A* substance. It should be noted that phosphotungstic acid forms an insoluble complex with potassium and has been used in the quantitative determination of potassium by Rieben and Van Slyke (1944). This does not mean that the phosphotungstic acid locates potassium specifically for it may react with numerous substances; nevertheless, the observed absorption of this stain in the *A* band is consistent with the conclusion that the *A* substance does contain a relatively high concentration of potassium. That the *A* substance is closely associated with the myosin filaments is indicated by the lack of any observable quantity of interfilamentary material in frayed *A* bands.

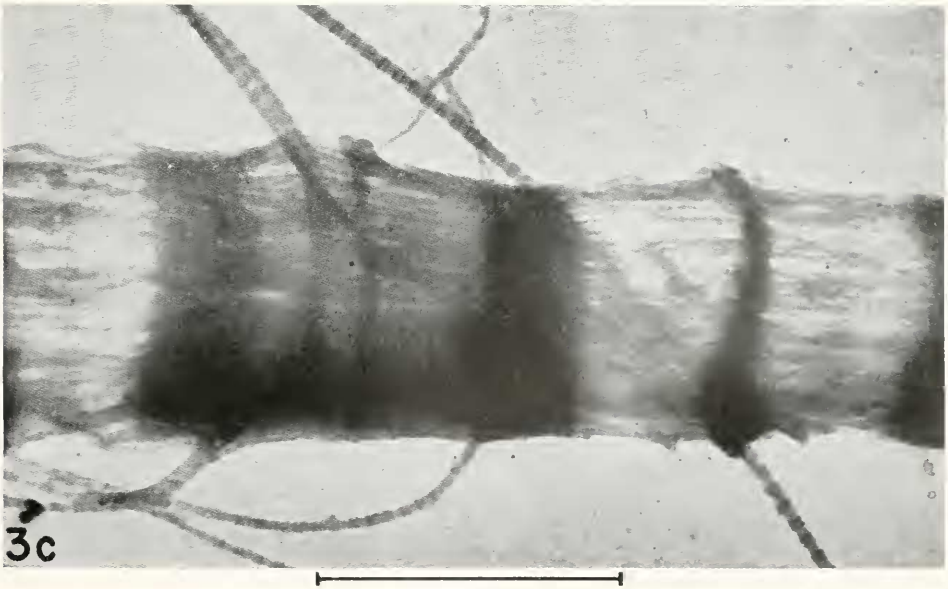
The sharp boundary between the *A* and *I* bands is noteworthy since there is no apparent membrane or other structure to confine the *A* substance. Owing to the limited resolution of the light microscope it had been concluded (Schmidt, 1937) that the transition between the *A* and *I* bands is not abrupt but gradual. Likewise there is no evidence in electron micrographs for any envelope or limiting membrane around the myofibril.

One of the most prominent histological features of striated muscle is the *Z* membrane, the nature of which has been the subject of much discussion in the past. In electron micrographs the *Z* membrane appears to be amorphous material of high staining affinity which cements the myosin filaments together in this region. Frequently in frayed myofibrils the filaments separate laterally but adhere to one another in the region of *Z* and also at *M*. Furthermore, there is a tendency for the filaments to break at *Z* and sometimes at *M*. It has been postulated that the *Z* membrane takes the form of an annular ring about the fibril (see Liang, 1936). Although this could be true, there is no unequivocal electron microscope evidence for such a conclusion. The *Z* membrane appears to consist of interfilamentary material present throughout the fibril and not limited to the periphery. It is definitely not collagenous as was suggested by Häggqvist (1931).

FIGURE 3. Myofibrils from frog sartorius, stretched about 30 per cent, stained with phosphotungstic acid; (*c*) shows collagen fibrils. × 40,000.

PLATE II





Collagen in muscle

In the preparation of specimens no effort was made to separate the collagen, which occurs in skeletal muscle in appreciable quantity. However, there is no difficulty in identifying collagen since it has a regular spacing of about 640 Å and displays a characteristic fine structure after staining with phosphotungstic acid (Schmitt, Hall, and Jakus, 1945). Several stained collagen fibrils are visible in Figure 3c. It has been observed that collagen fibrils from rabbit and frog muscle are quite uniform in width (about 500 Å), but the relation of this protein to the muscle structure is not evident in present electron micrographs because of the extensive fragmentation of the muscle.

Structural alterations in extension and contraction

Although evidence is not yet sufficient to permit a complete description of the structural alterations associated with extension and contraction, some observations have been made and may be described. Extended sarcomeres were obtained by stretching an excised frog sartorious and fixing it at the stretched length. In some instances the muscle was stimulated electrically during fixation to produce isometric contraction. In the absence of the electrical stimulus, the fixative itself provided a weakly stimulating effect. In other experiments, excised muscles were placed in formalin without restraint, thus producing a state of weak isotonic contraction. Strong isotonic contraction was produced by electrical stimulation of excised muscles before and during fixation. Myofibrils from these muscles were prepared for electron microscope observation in the manner described.

The high concentration of *A* substance toward the ends of the *A* band, resulting in the appearance of the *H* disc, occurs consistently in myofibrils from muscles which have been stimulated (electrically or by the fixative) while held in a state of extension (Figs. 1 and 3). Muscles which are free from tension and are stimulated to contract during fixation do not in general show a division of the *A* band. Myofibrils from such muscles are shown in Figure 2.

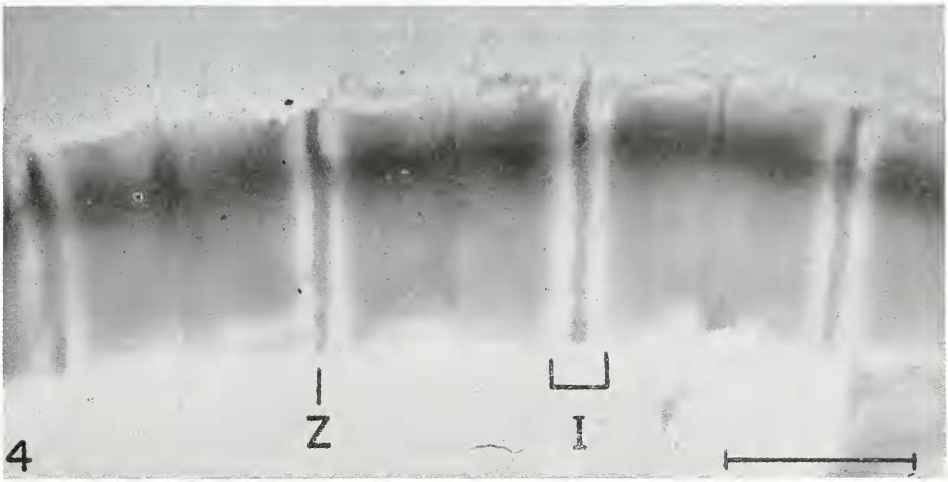
In the myofibrils of muscles which were stretched up to 130 per cent of rest length and fixed at this length, the *A* bands are about the same length as those in muscles which had contracted slightly as a result of fixation. Thus the increase in sarcomere length in stretched and fixed muscles is due mainly to an increase in the length of the *I* band. This observed relative constancy in the length of the *A* band during extension is in agreement with the findings of Buchtal, Knappeis, and Lindhard (1936) who concluded that the *I* band has a relatively low modulus of elasticity.

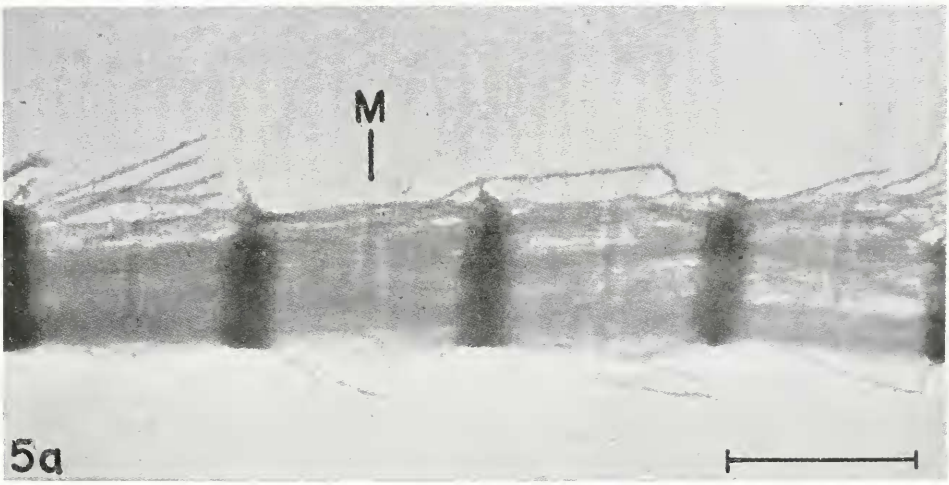
Electron micrographs of myofibrils from muscles fixed in a state of strong contraction show sarcomere lengths down to about 1 μ as compared with about 2 μ in relaxed or weakly contracted muscles. In general the observed sarcomere structure is of two types. The first type, shown in Figure 4, consists of a very narrow *I* band and a quite solid *A* band in which the median *H* disc is absent or very faint. The sarcomere length in Figure 4 is about 1.5 μ, which is about 25 per cent less than the average length in relaxed muscles. The second type of sarcomere structure

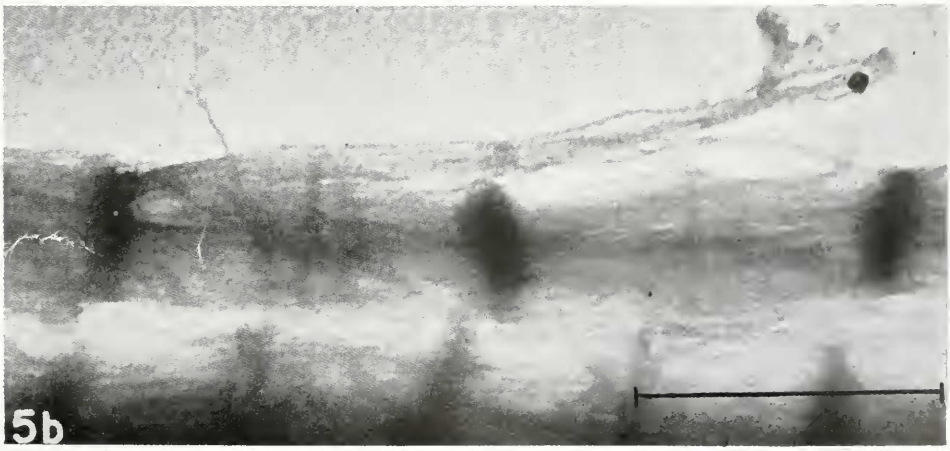
FIGURE 4. Myofibril from frog sartorius contracted by electrical stimulation, stained with phosphotungstic acid. × 25,000.

FIGURE 5. Myofibrils from frog sartorius, strongly contracted by electrical stimulation. (*a*) × 25,000 (*b*) × 40,000.

PLATE III







found in strongly contracted muscles is shown in Figure 5. Sarcomere lengths in this case are about 1.2 μ, which is about 60 per cent of the average relaxed length. The *I* band is no longer visible and *Z* appears somewhat wider and more poorly defined than in less strongly contracted fibrils. The region between *M* and *Z* is of uniform density and presumably contains a uniform concentration of *A* substance. In occasional fibrils the *Z* and *M* are distinguishable only with difficulty. Such fibrils may represent a transition stage between the two types of fibrils described. If this is the case, the second type of fibril might result from the accumulation, around the *Z*, of *A* substance which had migrated away from *M*. This stage apparently corresponds to the striation reversal described by Jordan (1933) and earlier by Rollett (1891), who refers to this densely staining *Z* as the *C*, or contraction band.

One aspect of particular significance in strongly contracted sarcomeres is the relative straightness of the myosin filaments, as seen in Figure 5. Since the contracted sarcomeres are as little as 50 per cent of the relaxed length, this can only mean that the filaments themselves shorten in contraction. Similarly, in extension the filaments must individually lengthen. Changes in sarcomere length are not to be associated with any gross spiralling or folding of filaments in the order of dimensions visible in the electron microscope. The myosin filaments are the smallest visible contractile units.

Isolated Myosin Filaments

Myosin may be extracted from muscle in weakly alkaline salt solutions and these extracts have been shown to contain filaments visible in the electron microscope (Ardenne and Weber, 1941). In order to determine whether visible differences exist between different myosins, a quantitative study of filaments from various muscles was undertaken.

Extracts of myosin were prepared by a method essentially similar to that described by Greenstein and Edsall (1940). Muscles were removed from the animal immediately after death, trimmed, cut into small pieces and blended in a Waring Blendor with about ten parts of a cold solution of KCl (0.5 M) and $NaHCO_3$ (0.03 M). The suspension was stirred mechanically, at about 4° C., for periods of time varying from seven to twenty-four hours and strained through several layers of closely-woven cheese-cloth. The filtrate, showing strong double refraction of flow, was poured into 8 to 10 volumes of cold distilled water, with constant stirring, and the precipitate which formed was allowed to settle overnight in the cold. Further concentration was accomplished by centrifugation, after removal of the supernatant. The precipitated myosin was washed with cold distilled water, and redissolved by adding powdered KCl crystals to a concentration of about 0.45 M. To further purify the myosin the precipitation and solution was repeated once or twice.

For electron microscope examination, the myosin preparation was diluted to the desired concentration with the KCl–$NaHCO_3$ solution used in the extraction and centrifuged to remove any undissolved protein. A droplet of the solution was

Figure 6. Myosin filaments from rabbit leg muscle. × 30,000.
Figure 7. Myosin filaments from lobster abdominal muscle. × 30,000.
Figure 8. Myosin filaments from clam (Mya arenaria) adductor muscle. × 30,000.

PLATE IV

placed on the supporting film of the specimen grid, the excess removed by blotting, and the film washed with Edsall's solution. The adhering filaments were then fixed and stained, for about one minute, with 0.1 per cent phospho-12-tungstic acid (pH 3 to 5) and washed briefly with water to remove the uncombined phosphotungstic acid. Unstained filaments are poorly defined because of low contrast and, in the absence of the stabilizing effect of the heavy metal ion, appear to be adversely affected by the drying process.

Myosin extracts were made from the muscles of rabbit leg, frog leg, lobster abdomen, scallop adductor (striated part) and clam (Mya) adductor (classified as smooth muscle). Electron micrographs of such myosin preparations show slender filaments similar to those observed in intact myofibrils and resembling those shown by Ardenne and Weber (1941). The filaments have varying lengths and widths and display no tendency to branch or split longitudinally. They are rough in appearance and show fluctuations in density along their length. However, there is no evidence of any regular structural variation which could be interpreted as a significant periodicity. There are no observable changes in density which can be correlated with the *A* and *I* bands, which indicates that the *A* substance has probably been washed out during extraction. This general appearance is very much the same for myosin filaments from the five different animal forms. Typical electron micrographs of myosin filaments from rabbit, lobster, and clam are shown in Figures 6, 7, and 8 respectively.

A statistical study of lengths and widths of myosin filaments from the five selected forms was made. Measurements were taken directly from enlarged prints and in any given print all filaments were measured which could be discerned as complete and individual. Widths can be measured only approximately because of the smallness of this dimension and also because of the roughness of contour. The uncertainty of measurement, however, is significantly smaller than the spread in widths. Results are shown in Figure 9. Each interval in the plot of widths contains the same number of measurable increments and each set of measurements represents about 300 filaments.

TABLE I

Dimensions of myosin filaments

Muscle source	Average width	Average length
Rabbit leg	120 Å	4100 Å
Frog leg	140 Å	4100 Å
Lobster abdomen	140 Å	6800 Å
Scallop adductor, striated	130 Å	5000 Å
Clam (Mya) adductor, smooth	150 Å	3100 Å

Nearly all measured widths fall into a narrow range between 50 and 300 Å. There may be some filaments having widths below 50 Å which were not observed because of the resolution and contrast limitations of the electron microscope. However, the quality of the background in the electron micrographs and the fact that all distributions fall off toward small widths indicate that no great quantity of the myosin occurs in this range in electron microscope specimens as prepared.

Between one animal form and another there is very little variation in magnitudes or distribution of widths. There is a slight difference in the averages as shown in Table I, but it is so small as to be scarcely significant.

Filament widths were also measured from electron micrographs of intact myofibrils from frog muscles. The distribution plot resembles very closely the corre-

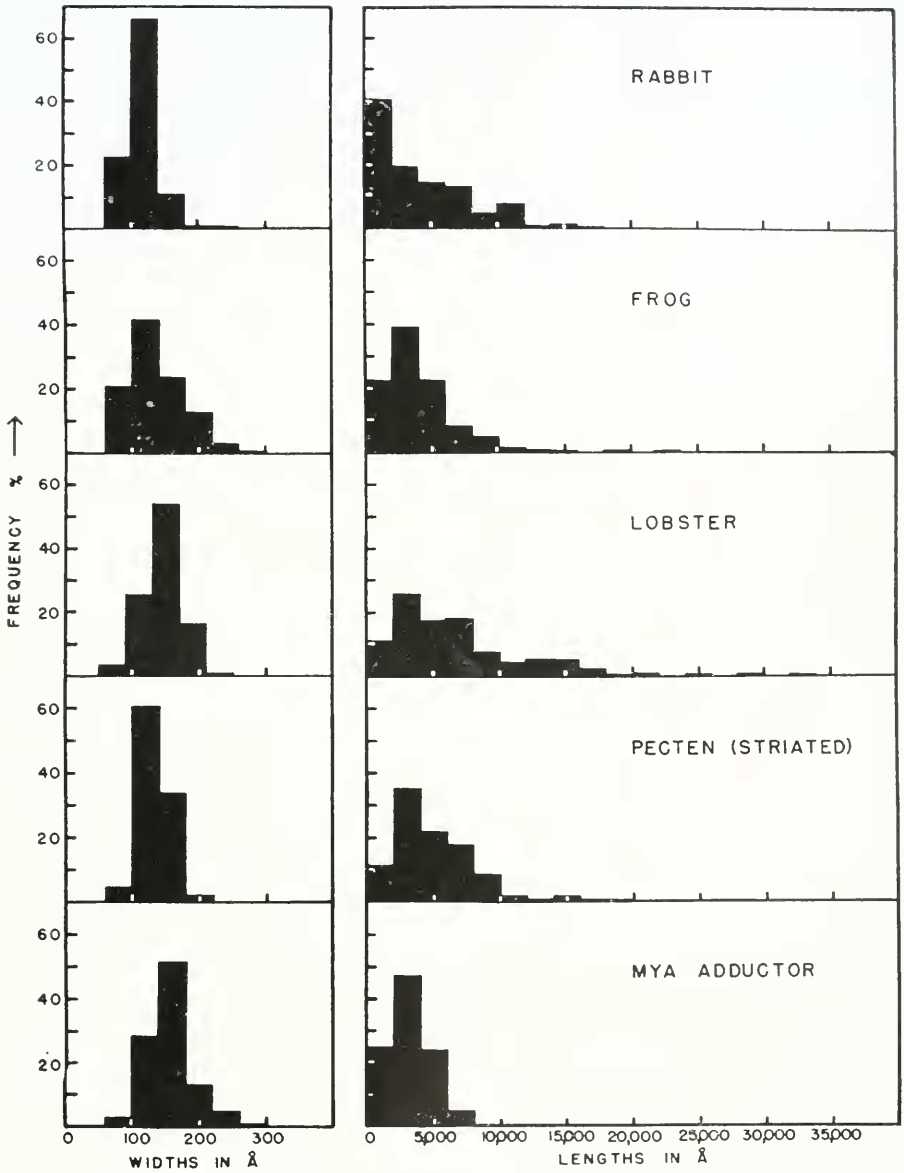


FIGURE 9. Distribution of lengths and widths of myosin filaments from muscles of various animal forms.

sponding plot for frog shown in Figure 9, thus indicating that there is no great difference in the width of filaments in electron micrographs of intact fibrils and myosin extracts.

In comparison to the fair constancy of widths there is a wide distribution of lengths. Most of the measured lengths are below 15,000 Å. Filaments from lobster muscle are in general significantly longer than the others, while filaments from Mya aductor, which is the only smooth muscle studied, are significantly shorter. As a result of the wide range of lengths there is only rough significance in the number-averages given in Table I. The distribution plots are reasonably reproducible under similar conditions.

It is evident from the statistical results that myosin extracts of this type do not constitute a monodisperse system and there is no justification for referring to the filaments as molecules. Widths of filaments apparently do not alter appreciably during extraction. The lengths, however, bear no relation to any observable dimensions in the intact myofibrils. Since most of the lengths represented in the plots of Figure 9 are considerably shorter than are the corresponding sarcomeres, it is apparent that the myosin filaments have been broken at random into shorter segments during the extraction procedure.

Since the myosin filaments seen with the electron microscope are to be identified with the asymmetrical particles responsible for the streaming double refraction of myosin solutions, it is pertinent to compare the results of the methods where possible. Mehl (1938) reported the length of rabbit myosin as 8,500 Å while Edsall (1942) gives a figure of 12,600 Å. The second figure is near the upper limit of the distribution plot for rabbit in Figure 9 while the first falls within the range of lengths representing the bulk of the protein. Although no figures have been reported on the other muscles used here, Edsall and Mehl (1940) have described lobster myosin as being more viscous than rabbit myosin at equal concentration and strongly birefringent, which is in qualitative agreement with the electron microscope observation that lobster preparations contain significantly longer fibrils than do those from rabbit.

Ardenne and Weber (1941) do not give the source of the myosin extract from which they made electron micrographs. They state that the filament widths are from 50 to 100 Å although no measurements are tabulated and the micrographs used as illustration contain filaments at least 200 Å in width. The myosin filaments within the limited field of the micrographs which these authors have shown are apparently similar to those described here.

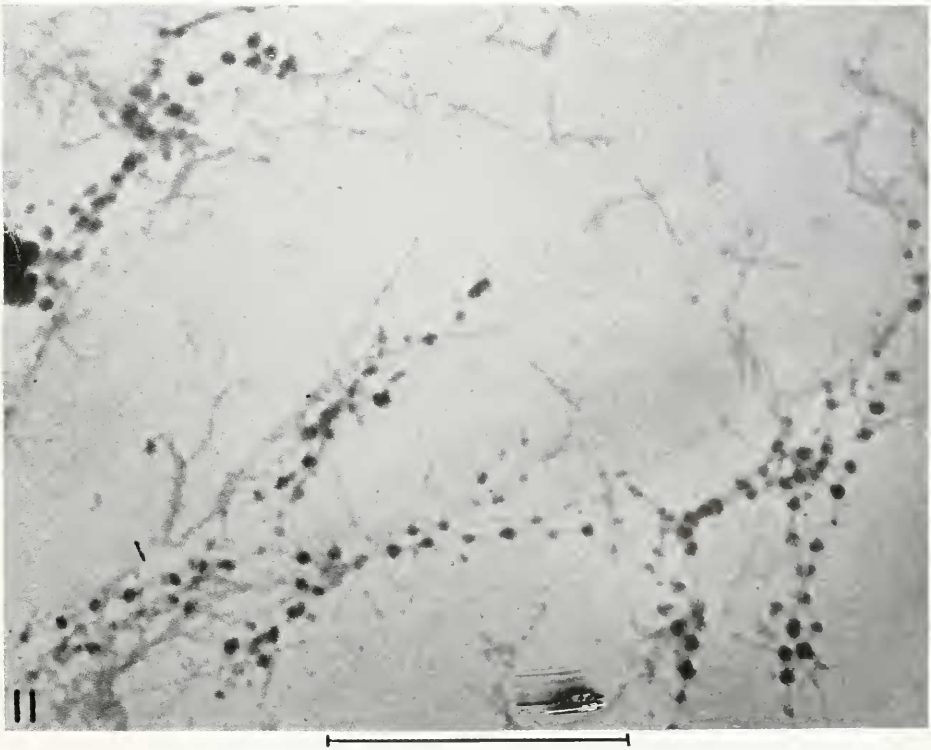
Paramyosin

In a recent publication Hall, Jakus, and Schmitt (1945) described the structure of a fibrous muscle globulin which is present in appreciable amount in molluscan smooth muscles, and correlated the observed structure with the x-ray patterns obtained by Bear (1944) from the same material. Since this protein can be identified by electron microscope observation and x-ray diffraction it merits a distinguishing name and is therefore designated as *paramyosin*. Although paramyosin

FIGURE 10. Regular structures from clam adductor muscle after dispersion in Edsall's solution. × 40,000.

FIGURE 11. Fragments from dispersed clam muscle. Darkly staining particles are adhering to fine filaments, presumably myosin. × 40,000.

PLATE V



resembles myosin in some respects, there are definite differences between the two proteins. Paramyosin contains a characteristic axis spacing of about 145 Å. Paramyosin has not been detected in striated muscle while myosin is apparently common to all muscles. Furthermore, the typical needle-shaped fibrils of paramyosin disintegrate in Edsall's solution (0.5 M KCl, 0.03 M $NaHCO_3$) while the myosin filaments remain essentially the same as in fixed intact muscle except as they may be broken into shorter lengths during extraction.

Clam adductor muscles

The adductor muscle of the clam differs from the other muscles investigated in that it is classified histologically as "smooth" muscle and contains a large quantity of the fibrous protein, paramyosin. Electron microscope observation has failed to reveal any large periodicity resembling the characteristically banded sarcomere of striated muscle. However, if clam muscle is dispersed in Edsall's solution and centrifuged, there are thrown down fibrous aggregates to which large particles adhere. A typical micrograph of this type of material is shown in Figure 10. The structure consists essentially of bundles of fine filaments (presumably myosin) and darkly staining nodules producing a cross striation with a period of about 1,100 Å. In Figure 11 the bundles have been dispersed, revealing the individual dark nodules which adhere to the fine filaments at more or less regular intervals. The attachment of the dense component at regular intervals along myosin bundles (Fig. 10) is suggestive of a rudimentary structure analogous to the *Z* membrane and sarcomere of striated muscle. As yet, the significance of this structure is not known.

Discussion

With respect to the existence and disposition of the cross striations, the electron microscope observations on fixed muscle, stained and unstained, are in close agreement with the results obtained with the light microscope (see Jordan, 1933). Confirmatory evidence is presented concerning the *N* bands, *H* discs and the nature of "striation reversal" in strong contraction. Additional information has been added by virtue of the high resolution of the electron microscope. Thus it is possible to say definitely that the *Z* band is not collagenous as suggested by Häggqvist (1931) and to observe directly the myosin filaments and their relation to previously determined structures.

Of fundamental importance is the observation that the myosin filaments extend continuously through the fibril in relatively parallel straight lines. There is no marked disorientation of the filaments in either *A* or *I* bands of fibrils in any state of contraction. Although the optical anisotropy of the *A* band led to the postulate that it contains asymmetric myosin particles, there has always been some doubt as to whether the relatively isotropic *I* band consists of myosin in unoriented state or of some other protein (Weber, 1934). It is now clear that the *I* band consists mainly of well oriented myosin filaments and it is not possible to account for the low birefringence on the basis of gross disorientation. Possibly the difference in anisotropy between the *A* and *I* bands is to be attributed to differences in orientation within the filaments. The only obvious alternative explanation is that the low birefringence in the *I* band is due to partial compensation of the birefringence of the myosin filaments by other components.

No evidence has been found in this investigation for the existence, either in myofibrils or in extracted myosin, of "rodlets" of the specific dimensions postulated by Weber (1934) from polarized light and diffusion experiments. Theoretical difficulties underlying Weber's calculations have been pointed out by Frey-Wyssling (1940) and Schmitt (1944).

Electron micrographs of isolated myosin filaments show that in width and general appearance the filaments are similar to those seen in fixed intact myofibrils. Although myosin filaments from whatever source are essentially the same, the statistical study indicates that they occur in various lengths and are not to be designated as discrete "myosin molecules". However, the length of rabbit myosin reported by Mehl (1938) from streaming double refraction studies is reasonably close to the weight-average length calculated from the distribution curve in Figure 9. No reliable measurement of width is available for comparison with the electron microscope results.

Since myosin filaments in intact fibrils are continuous, the wide range of lengths found in myosin suspensions is noteworthy. It appears that the filaments, while little changed in width, are broken more or less at random during the extraction procedure. The longest filaments approach the sarcomere length and there may be significance in the fact that lobster muscle yields longer filaments than does frog muscle and also has the longer sarcomere. The absence of filaments longer than their corresponding sarcomere is consistent with the observed tendency of the filaments to break at *Z*.

Ziff and Moore (1944), following an extraction procedure similar to that used here, state that their myosin solutions contain a homogeneous substance which forms sharp boundaries in electrophoresis and sedimentation. If homogeneity is meant to denote constancy in particle length, this conclusion is in disagreement with the electron microscope results. In considering the apparent inconsistency it should be noted that, in the electrophoresis and sedimentation of rod-shaped particles of nearly constant diameter, a sharp boundary does not necessarily indicate a constant length. Schramm and Weber (1942) reported that a small fraction of the myosin in extracts has a much higher sedimentation constant than that of the predominant component. No evidence for such a distinct heavy component has been found in the present study unless it represents aggregates of myosin filaments.

The "molecular weight" of myosin has been estimated by Weber and Stöver (1933) to be $0.6 - 1.2 \times 10^6$ and by Ziff and Moore (1944) to be 3.9×10^6. If a particle weight is calculated from the average dimensions of the filaments in rabbit myosin from Table I, using a density of 1.3 and assuming a circular cross section, the result is 36×10^6. The results differ by at least a whole order of magnitude. In view of the fair agreement as to filament lengths from streaming birefringence and electron microscope observations, the discrepancy in particle weight is difficult to understand. It may be that the cross section of filaments dried on the supporting film is not circular as assumed in the electron microscope calculation, but it is doubtful whether this assumption could introduce an error large enough to account for the discrepancy. In any event, the significance of all such calculations is questionable inasmuch as the extracts contain particles of widely differing dimensions, in no sense to be considered as molecular entities.

Bear has reported small-angle x-ray diffractions from various muscles. One set of diffractions (Bear, 1944) has been correlated with the structure of para-

myosin fibrils, as determined from electron micrographs (Hall, Jakus, and Schmitt, 1945). This set of diffractions has not been obtained from any striated muscle.

In addition Bear (1945) has reported a second set of small-angle diffractions obtained from a variety of muscles, both smooth and striated. The wide occurrence of this pattern is strongly suggestive that the diffractions originate in the myosin component but it has not been possible to identify in the electron microscope the structure responsible for the diffractions. Furthermore, since the meridional diffractions are orders of 27 Å, it is doubtful whether the structure can be observed directly. Another feature of the diffractions is the occurrence of a periodicity estimated by Bear to be between 350 and 420 Å. Although this dimension is quite large enough for electron microscope resolution, it appears that the pattern is somewhat like the paramyosin pattern in that the large periodicity cannot be discerned unless the axis spacing (27 Å) is well resolved. The fine banded appearance frequently observed in myofibrils is of about the same order of magnitude as the large periodicity, but this may be fortuitous. The x-ray data also indicate a lateral periodicity of about 115 Å which should be large enough for electron miscroscope observation. Although the *average* width of myosin filaments is quite close to this figure, the significance, if any, of this coincidence remains to be determined.

Since the myosin filaments are observed to pursue a straight course through the *A* and *I* bands in fibrils from contracted as well as relaxed muscles, it may be concluded that contractility is a property of the individual filaments in their normal environment in muscle. It seems probable that alterations in length and tension depend on changes within the filaments in response to changes in the chemical environment. In seeking a description of the contractile mechanism, due consideration must be given to the role played by the *A* substance and the adenosine triphosphate. The electron microscope technique provides a promising method for studying these structures and processes. It may be expected that further correlation of the x-ray, electron microscope, polarization optical and chemical evidence will contribute greatly to an understanding of the nature of contractility.

Summary

1. Electron micrographs were made of myofibrils isolated from frog and other skeletal muscles fixed in formalin. The structure with respect to the location and disposition of the principal cross striations is in good agreement with that previously determined from histological studies.

2. The myofibrils are composed of bundles of myosin filaments ranging in width from about 50 to 250 Å and extending continuously and in relatively straight lines through the isotropic and anisotropic bands in both the extended and contracted states. The anisotropic bands also contain material of high electron scattering power and affinity for phosphotungstic acid. The distribution of this "*A* substance" changes with contraction in characteristic fashion. The evidence indicates that the myosin filaments are the contractile units.

3. While the myosin filaments have an indefinite length in the intact fibril, they are fragmented extensively during extraction in weakly alkaline salt solutions (method of Greenstein and Edsall). Filaments from such extracts have fairly uniform widths (50 to 250 Å) but highly variable lengths, in general below 15,000 Å. Filaments from rabbit, frog, lobster, scallop and clam muscles are similar in appear-

ance; widths are fairly uniform but lengths vary significantly from one form to another.

4. The relation of these findings to physical chemical data previously obtained by others on similar myosin extracts is discussed.

5. In the one smooth muscle examined (clam adductor) no striations comparable to those of skeletal muscle fibrils were found. However, a regular structure with a period of about 1,100 Å was observed.

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